

QUANTITATIVE STUDIES IN ION ANTAGONISM

I. EXPERIMENTS ON THE STRIATED MUSCLE OF THE FROG

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Although the study of ion antagonism goes back to the year 1882 when Ringer published his first paper on ion antagonism in the heart, quantitative study of the subject has just begun. Loeb (1915*b*) suggested the interesting theory that the quantitative relationship in ion antagonism depends upon the function concerned. If ions influence irritability, a linear relation is supposed to exist between the antagonistic ions, while a parabolic relation expresses the antagonism if permeability is concerned. Recently Rubinstein (1928) performed experiments on a polychæte worm (*Fabricia sabella*) and found a parabolic relationship $[K] = a\sqrt{[Na]}$, although there was no proof that this antagonistic action was based upon changes in permeability. It is the writer's opinion that a sharp distinction between the two groups as suggested by Loeb is not possible at the present time. Changes in the behavior of the surface of cells may often initiate changes in permeability (Gellhorn, 1930). In spite of this, the ingenious idea of Loeb, that the quantitative study of ion antagonism may reveal different kinds of antagonism and may finally lead to a better understanding of that problem, merits further investigation. In contrast to the work of Loeb (1915*a*) on *Balanus* larvæ and that of Rubinstein (1926) on *Fabricia*, in which several tissues were involved, the writer has considered it preferable to examine ion antagonism as it affects one single structure. This is possible in muscle, for Kato (1929) has shown in histological studies that the antagonistic ions act upon the same anatomical structure.

It is apparent from the investigations of Overton (1904) that the irritability of striated muscle depends chiefly upon NaCl, KCl, and CaCl₂. Therefore a quantitative study of ion antagonism was carried out between these ions. The effect of the trace of NaHCO₃ added to the Ringer's solution to preserve neutrality was not taken into consideration.

METHOD

The experiments were chiefly performed on the sartorius muscle of *Rana esculenta* between January and April, 1930. Frogs of medium size, which did not vary more than 10 per cent in weight, were chosen. The sartorii were prepared carefully in the usual manner and suspended between platinum electrodes. The muscles were kept in the salt solution, which was aerated with purified oxygen. At intervals of about thirty minutes the threshold of the muscle was determined

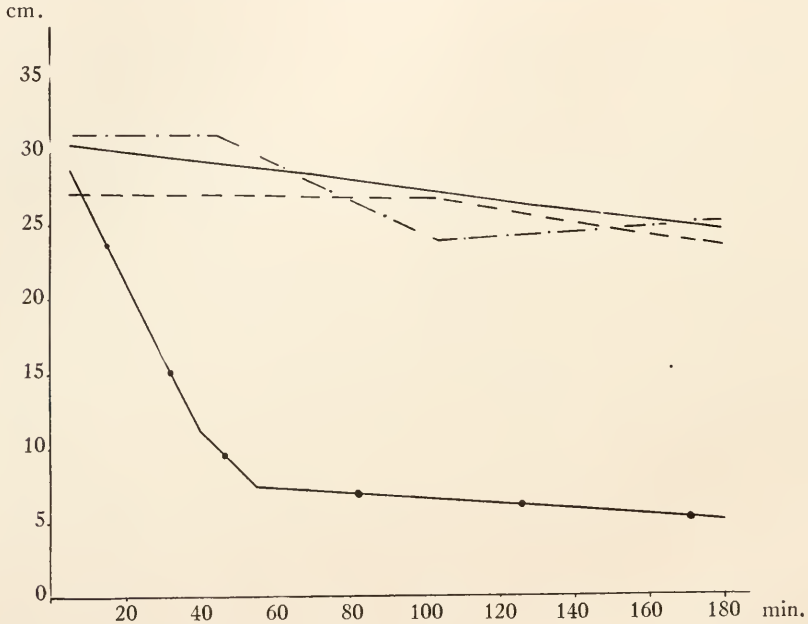


FIG. 1. The influence of the variation of KCl—NaCl and CaCl_2 being constant—upon the irritability of the sartorius.

Ordinate: threshold in cm. distance between primary and secondary coil.

Abscissa: time in minutes.

0.042 M NaCl + $8.1 \text{ M} \times 10^{-4} \text{ CaCl}_2$ + $11.3 \text{ M} \times 10^{-4} \text{ KCl}$ —————
 " " " + $17 \text{ M} \times 10^{-4} \text{ KCl}$ - - - - -
 " " " + $20 \text{ M} \times 10^{-4} \text{ KCl}$ - · - · -
 " " " + $23 \text{ M} \times 10^{-4} \text{ KCl}$ —●—●—●—

after having removed the muscle from the liquid. The interrupted current of an induction coil, the primary circuit of which was supplied with one dry cell (1.5 volts), was used. In a series of preliminary experiments it was found that if at constant NaCl and CaCl_2 content the influence of increasing KCl concentration was investigated during the observation time of three hours, a sharp distinction between two groups of KCl effects could be made. In the first group, containing

the lower KCl concentration, the threshold increased very gradually within three hours and the final value was indicated by a distance of at least 20 cm. between primary and secondary coils. In the greater concentrations, however, the irritability decreased very rapidly and fell far below 20 cm. The initial value, which varied between 25 and 35 cm. distance, had no influence on this typical behavior. Thus it was possible to determine exactly the threshold of the KCl effect which just leads to a loss of irritability below 20 cm. distance. In about 200 experiments, there were very few cases where the classification was doubtful. In such cases several repetitions of the experiments gave decisive results. A typical example illustrating the accuracy of the method is reproduced in Fig. 1. Further on it is shown in Fig. 2 that the antagonistic action of CaCl_2 can be determined exactly in the same manner.

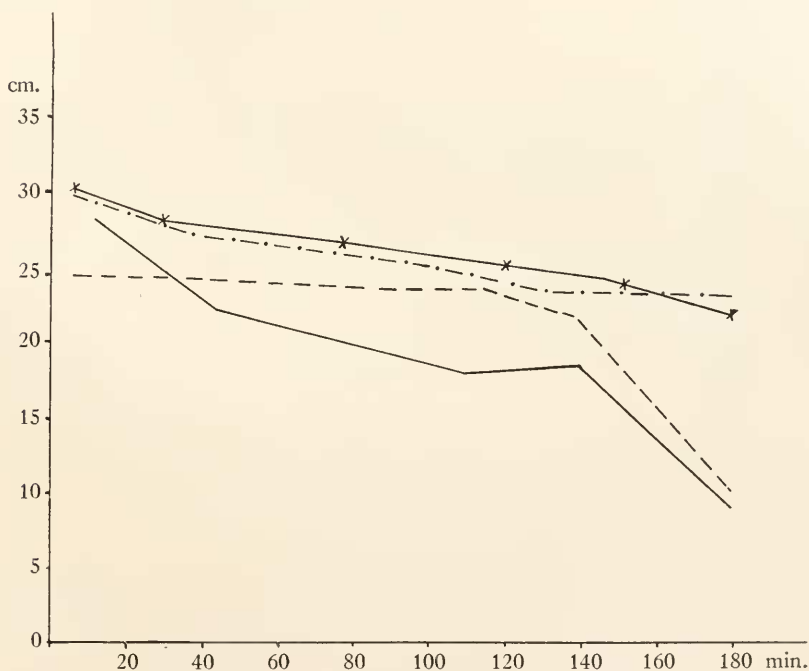


FIG. 2. The influence of variation of CaCl_2 —NaCl and KCl being constant—upon the irritability of the sartorius.

Ordinate and abscissa as in Fig. 1.

0.084 M NaCl + 84 M $\times 10^{-4}$ KCl + 46.57 M $\times 10^{-4}$ CaCl_2	—————
“ “ “ 48.6 “	-----
“ “ “ 50.62 “	- - - - -
“ “ “ 52.65 “	-x-x-x-

The solutions used had a freezing point of $\Delta = -0.39$. They were 0.113 M NaCl, 0.113 M KCl and 0.081 M CaCl_2 . Greater concentrations of NaCl were obtained with different amounts of 1 per cent NaCl. The amount of liquid used was always 20 cc.

I. *The Antagonism between Na and K*

In a series of experiments the dependence of the KCl effect upon the NaCl concentration was studied. The CaCl_2 concentration, $8.1 \text{ M} \times 10^{-4}$, was constant in all experiments. There were two ways of varying the NaCl concentration: first, by replacing a part of the NaCl solution by isotonic glucose solution, thus avoiding changes in the osmotic pressure for all solutions containing less NaCl than corresponds to the isotonic concentration; second, without the

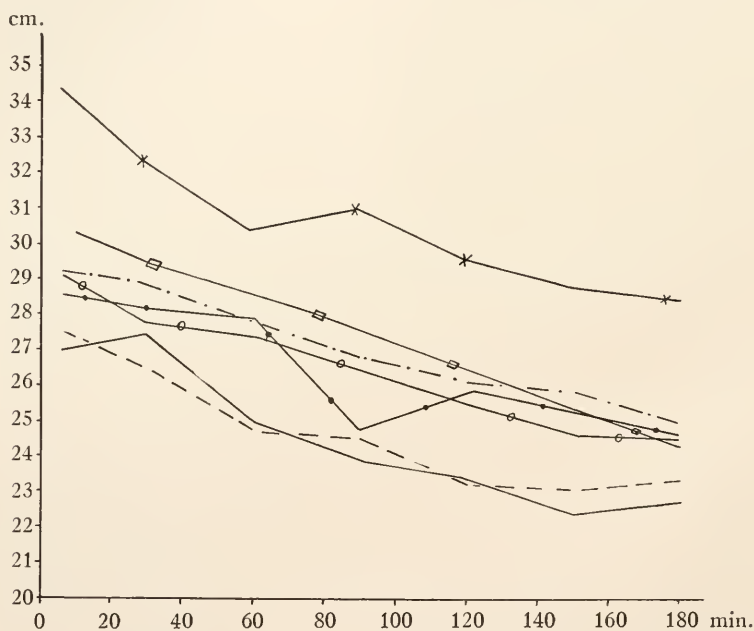


FIG. 3. The influence of variation of NaCl—KCl and CaCl_2 being constant—upon the irritability of the muscle. Each solution contains $5.65 \text{ M} \times 10^{-4}$ KCl + $8.1 \text{ M} \times 10^{-4}$ CaCl_2 .

Ordinate and abscissa as in Fig. 1.

0.042 M NaCl	—□—□—
0.056 M “	— — — — —
0.071 M “	— — — — —
0.08 M “	—x—x—
0.09 M “	—o—o—
0.113 M “	— · — · —
0.127 M “	—●—●—

addition of any non-electrolytes. The experiments were performed in both ways and it was found that glucose even in small amounts in the presence of NaCl is not indifferent to the muscle. The effective minimal concentration of KCl was greater with the addition of glucose than without it. This shows that glucose protects the muscle to a certain extent from the harmful effect of KCl. Similar observations were made in a study of the KCl contracture in muscle (Gellhorn, 1926). Because the osmotic pressure can be maintained constant for various concentrations of NaCl only by the addition of varying amounts of non-electrolytes, the effective minimal concentration of KCl would also be dependent upon the sugar concentration. In order to avoid these complications, salt solutions without non-electrolytes were used in spite of differences in the osmotic pressure. It is apparent from Fig. 3 that it was possible to study the effect of NaCl concentration between the values 0.042 M and 0.127 M because the threshold of the muscle remained above 20 cm. for three hours in spite of the osmotic differences.

Table I contains data from a series of experiments in which the dependence of the minimal effective KCl concentration upon the NaCl concentration was studied. The experiments show the great accuracy with which this value can be determined. It is:

Concentration KCl ($M \times 10^{-4}$) for M NaCl	
23.....	0.042
43.....	0.056
51.....	0.071
68.....	0.092
85.....	0.113

One recognizes that with increasing NaCl concentration the KCl concentration must also increase in order to become effective. There is therefore an antagonism between NaCl and KCl. The quantitative relationship is to be seen in Fig. 4. It shows that there is a linear dependence of $[K^+]$ upon $[Na^+]$ within the limits of 0.049 M NaCl and 0.113 M NaCl. Only in the extreme NaCl concentrations are small deviations from the straight line observed. They are probably due to a greater sensitivity of the muscle to KCl because of the extremely abnormal osmotic pressure. Therefore, further experiments were restricted to the range in which the linear dependence is exactly correct. According to these experiments the ratio of Na to K is constant as may be seen from Table II.

TABLE I
The Dependence of the KCl Effect upon the NaCl Concentration

Group I. 0.042 M NaCl + 0.00081 M CaCl ₂						
M × 10 ⁻⁴ KCl concentration	5.7	11.3	17	20	23	26
Threshold in cm. after 5'	28.0*	30.4	27.1	31.1	29.0	26.4
“ “ “ “ 180'	21.8	24.6	23.5	24.7	<u>7.3</u> †	<u>12.0</u>
Group II. 0.056 M NaCl + 0.00081 M CaCl ₂						
M × 10 ⁻⁴ KCl concentration		34.0	40	43	45	
Threshold in cm. after 5'		29.4	31.3	31.0	31.4	
“ “ “ “ 180'		26.9	23.0	<u>6.3</u>	<u>7.9</u>	
Group III. 0.071 M NaCl + 0.00081 M CaCl ₂						
M × 10 ⁻⁴ KCl concentration	48	50	51	54	57	68
Threshold in cm. after 5'	31.5	35.5	34.5	32.0	32.1	36.5
“ “ “ “ 180'	22.2	21.8	<u>14.5</u>	<u>4.5</u>	<u>7.5</u>	<u>5.7</u>
Group IV. 0.092 M NaCl + 0.00081 M CaCl ₂						
M × 10 ⁻⁴ KCl concentration		62	65	68	71	
Threshold in cm. after 5'		27.4	27.3	27.0	26.8	
“ “ “ “ 180'		26.0	20.5	<u>9.0</u>	<u>7.5</u>	
Group V. 0.113 M NaCl + 0.00081 M CaCl ₂						
M × 10 ⁻⁴ KCl concentration		79	82	85		
Threshold in cm. after 5'		36.5	27.2	27.5		
“ “ “ “ 180'		23.2	20.5	<u>9.0</u>		

* The numbers represent the threshold of the muscle measured by the distance between primary and secondary coil.

† The underlining indicates that the threshold has fallen below 20 cm. distance.

TABLE II
Ratio of Na to K for Different Na Concentrations

M NaCl . .	0.042	0.049	0.056	0.071	0.085	0.092	0.099	0.113	0.127
$\frac{M \text{ NaCl}}{M \text{ KCl}}$. .	1.87	1.33	1.39	1.36	1.36	1.35	1.34	1.34	1.41

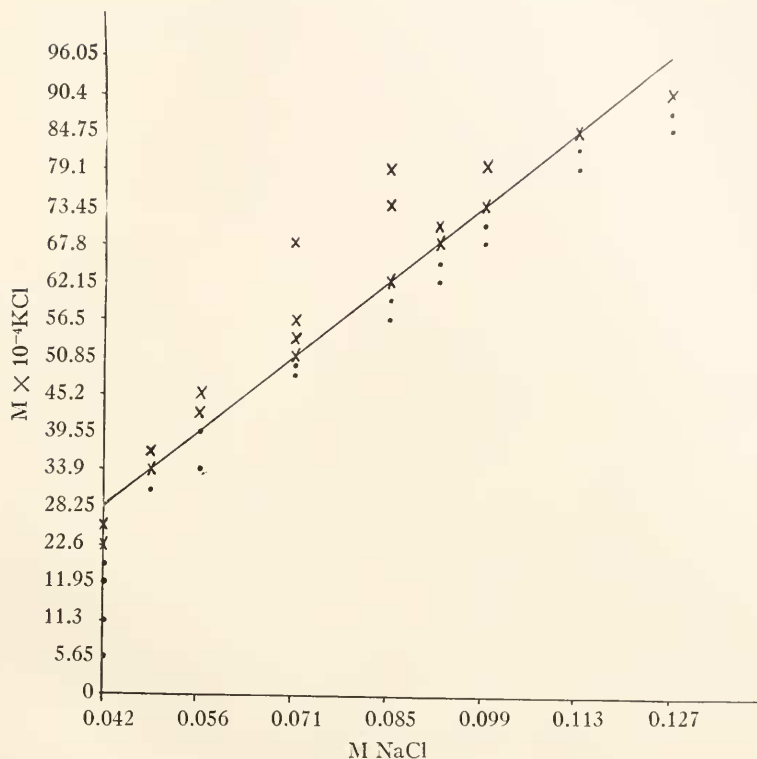
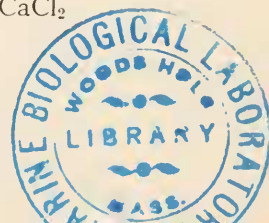


FIG. 4. The influence of the Na concentration upon the K effect.
 Abscissa: NaCl concentration.
 Ordinate: KCl concentration.

x = Rapid loss of irritability.
 . = Preservation of irritability.

II. *The Antagonism between K and Ca*

The solutions, the effects of which on irritability of the sartorius muscle were detailed in Part I, contained $8.1 \text{ M} \times 10^{-4} \text{ CaCl}_2$. Because it is known that Ca is the antagonist of K in muscle, the K effect in our experiments was balanced by $\text{NaCl} + \text{CaCl}_2$. In order to learn the quantitative relationship between K and Ca, the KCl concentration was varied and that CaCl_2 concentration which just sufficed to maintain the irritability above the 20 cm. threshold was determined. In these experiments the NaCl concentration was constant (0.071 mol.). As shown in Fig. 4, $8.1 \text{ M} \times 10^{-4} \text{ CaCl}_2$ was able to equilibrate the effect of $50 \text{ M} \times 10^{-4} \text{ KCl}$, but at the same CaCl_2 concentration $51 \text{ M} \times 10^{-4} \text{ KCl}$ led to a rapid decrease in irritability. In a series of experiments the corresponding CaCl_2



concentrations were determined when the solution contained $68 \text{ M} \times 10^{-4}$ KCl and $85 \text{ M} \times 10^{-4}$ KCl. The results were as follows:

TABLE III

The Ca → K Antagonisms in 0.071 mol. NaCl Solution

$\text{M} \times 10^{-4}$ KCl.....	50	68	85
$\text{M} \times 10^{-4}$ CaCl ₂	8.1	40.5	72.9

As Table III shows, the increasing concentration of KCl requires an increasing concentration of CaCl₂. Hence a linear relationship seems to exist between the two salts, since equal amounts of CaCl₂ above the basic value of $8.1 \text{ M} \times 10^{-4}$ antagonize approximately equal increases of KCl. But there is a very characteristic limit for this ratio. A KCl concentration which is higher than $91 \text{ M} \times 10^{-4}$ cannot be balanced even with the greatest concentration of CaCl₂.

On the other hand, further investigations showed that the antagonistic action of CaCl₂ against KCl is relatively greater below $8.1 \text{ M} \times 10^{-4}$ CaCl₂ than it is above it. Between 50 and $80 \text{ M} \times 10^{-4}$ KCl about $32.4 \text{ M} \times 10^{-4}$ CaCl₂ is able to offset the effect of $17 \text{ M} \times 10^{-4}$ KCl. That corresponds to the relationship, 0.001 M CaCl₂ to $5.25 \text{ M} \times 10^{-4}$ KCl. But as Table IV shows, below $8.1 \text{ M} \times 10^{-4}$ CaCl₂ the relationship is 0.001 M CaCl₂ to $27.7 \text{ M} \times 10^{-4}$ KCl.

TABLE IV

The Ca → K Antagonism in 0.07 mol. NaCl Solution

$\text{M} \times 10^{-4}$ KCl conc.....	34	39	44	50
$\text{M} \times 10^{-5}$ CaCl ₂ conc.....	20.25	40.5	60.75	81

These data lead to the conclusion that the Ca → K antagonism is represented by two straight lines forming an angle with each other. However, as Tables III and IV indicate, there is no constancy of the ratio of K to Ca when the CaCl₂ concentration is either below or above $8.1 \text{ M} \times 10^{-4}$. The difference in the quantitative behavior of the Na → K antagonism and that between Ca → K can be expressed mathematically. The equation of the straight line $y = ax + b$ holds for both antagonisms, but since in the former case b equals 0, $a = y/x = \text{const.}$, while in the Ca → K relationship b has a negative value. The constants of the experiments of Table III are $a = 1.9$, $b = -87.9$; those of Table IV, $a = 0.36$, $b = -9.7$.

Summing up these results and those of Part I, it may be said that Na and Ca ions are antagonistic to K. Leaving out of consideration

the case in which the CaCl_2 concentration is below $8.1 \text{ M} \times 10^{-4}$, the data show that $11.3 \text{ M} \times 10^{-4} \text{ KCl}$ can be equilibrated by $20.25 \text{ M} \times 10^{-4} \text{ CaCl}_2$. Furthermore, it is apparent from Fig. 4 that 0.014 M NaCl balances $11.3 \text{ M} \times 10^{-4} \text{ KCl}$.

III. *The Theoretical Calculation of the Sodium, Potassium, and Calcium Antagonism and its Experimental Proof*

It was pointed out that the results showed the relationship $0.014 \text{ M NaCl} \rightarrow 11.3 \text{ M} \times 10^{-4} \text{ KCl}$ and $20.25 \text{ M} \times 10^{-4} \text{ CaCl}_2 \rightarrow 11.3 \text{ M} \times 10^{-4} \text{ KCl}$ in which the arrow indicates that the first salt offsets the effect of the second one. On the other hand, the experiments reproduced in Fig. 4 show the minimal effective KCl concentration for different concentrations of NaCl in the presence of $8.1 \text{ M} \times 10^{-4} \text{ CaCl}_2$. Therefore, if the suppositions are correct, it should be possible to calculate the minimal effective KCl concentration if either the NaCl or the CaCl_2 concentration or both vary.

A series of experiments was performed in order to check the theoretical calculations and experimental facts. The experimental results are reproduced in Table V. The composition of the solutions is calculated according to the data described above. The CaCl_2 concentration of these solutions is supposed to be just insufficient to offset the KCl effects. Therefore it was expected that the addition of a small amount of CaCl_2 would warrant the maintenance of irritability above the 20 cm. threshold. Solutions which had this effect are marked with +, solutions causing rapid loss of irritability below 20 cm. threshold are marked with -. The calculated and the experimentally determined values check very well and show again that a linear dependence of KCl upon NaCl and CaCl_2 is characteristic of the ion antagonism in striped muscle.

In order to check these experiments still another series of experiments was performed on the gastrocnemius muscle of *Rana esculenta*. The muscle weight varied between .45 gram and .50 gram. Because these muscles are larger and the specific surface smaller, it was to be expected that they would be less sensitive to KCl than the sartorius muscles with their large specific surface. Correspondingly, it was found necessary to extend the time of observation to four hours in order to find differences in the irritability. Under these conditions the concentration of KCl was determined which just led to a marked decrease in irritability if NaCl and CaCl_2 were constant. This is a typical example:

M NaCl.....	0.084	0.084	0.084	0.084
$\text{M} \times 10^{-4} \text{ KCl}$	68	71	74	80
$\text{M} \times 10^{-4} \text{ CaCl}_2$	18.23	18.23	18.23	18.23
Threshold after 4 hours, in cm.....	31.6	32.6	23.8	11.0

TABLE V

The Antagonism between Na, K and Ca

+ = maintenance of irritability above the 20 cm. threshold.
 - = rapid decrease of irritability; threshold below 20 cm.

					M × 10 ⁻⁴ CaCl ₂	
					Observed	Calculated
Group I. 0.056 M NaCl + 0.0051 M KCl						
M × 10 ⁻⁴ CaCl ₂	28.35	30.38	32.4		28.35	28.35
Irritability	-	+	+			
Group II. 0.056 M NaCl + 0.0068 M KCl						
M × 10 ⁻⁴ CaCl ₂	58.73	60.75	62.78		60.75	58.73
Irritability	-	-	+			
Group III. 0.084 M NaCl + 0.0068 M KCl						
M × 10 ⁻⁴ CaCl ₂	16.2	18.23	20.25		16.2	18.23
Irritability	-	+	+			
Group IV. 0.084 M NaCl + 0.0085 M KCl						
M × 10 ⁻⁴ CaCl ₂	46.57	48.6	50.62	52.65	48.6	48.6
Irritability	-	-	+	+		
Group V. 0.092 M NaCl + 0.0085 M KCl						
M × 10 ⁻⁴ CaCl ₂	36.45	38.48	40.5		36.45	38.48
Irritability	-	+	+			
Group VI. 0.098 M NaCl + 0.0085 M KCl						
M × 10 ⁻⁴ CaCl ₂	28.35	30.38	32.4		28.35	28.35
Irritability	-	+	+			
Group VII. 0.098 M NaCl + 0.0102 M KCl						
M × 10 ⁻⁴ CaCl ₂	52.65	56.70	60.75	64.80	52.65	58.70
Irritability	-	+	+	+		
Group VIII. 0.098 M NaCl + 0.0097 M KCl						
M × 10 ⁻⁴ CaCl ₂	40.5	44.55	48.6	52.65	40.5	48.6
Irritability	-	+	+	+		
Group IX. 0.113 M NaCl + 0.0097 M KCl						
M × 10 ⁻⁴ CaCl ₂	24.3	28.35	32.4		24.3	28.35
Irritability	-	+	+			

In this series 74 M × 10⁻⁴ KCl was the smallest concentration which was effective. In the same way, the KCl concentrations in the presence of different amounts of NaCl and CaCl₂ which led to the threshold below 25 cm. distance between primary and secondary coils were determined. The results have been reproduced in Table VI.

They indicate that the calculated values are generally a little smaller than the observed ones, but that this is almost a constant error, showing that the same relations hold as well for the gastrocnemius as for the sartorius.

TABLE VI

The Antagonism between Na, K, and Ca (Experiments on Gastrocnemius)

No.	M NaCl	M $\times 10^{-4}$ CaCl ₂	M $\times 10^{-4}$ KCl	
			Observed	Calculated
1	0.056	28	54	51
2	0.056	59	71	68
3	0.071	18	57	57
4	0.071	49	79	76
5	0.084	18	74	68
6	0.084	49	91	85
7	0.092	38	91	85
8	0.098	28	88	85
9	0.098	49	99	97
10	0.113	28	102	97

IV. *The Limits of the Antagonistic Efficiency of CaCl₂*

In the last paragraph the possibility of calculating the minimal effective KCl concentration for different amounts of NaCl and CaCl₂ was indicated and a fairly good agreement was shown between experimental and calculated values. In these experiments the difference between the effect of NaCl and CaCl₂ in antagonizing KCl was of quantitative nature. But further experiments showed that this holds only within certain limits because the maximal concentration of KCl which can be balanced by CaCl₂ is determined by the concentration of NaCl. In Table VII it is shown that the maximal concentration of KCl which could be balanced by CaCl₂ varied from 71 to 108 M $\times 10^{-4}$ if the NaCl concentration varied.

TABLE VII

Limits of the Antagonistic Action of Ca toward K in Dependence upon the NaCl Concentration

M NaCl concentration	Maximal tolerated K concentration (M $\times 10^{-4}$)
0.056	71
0.071	91
0.084	102
0.098	108

The experiments make it probable that NaCl, although comparatively weaker than CaCl₂ in antagonizing KCl, exerts in part a specific influence. In this respect it cannot be replaced by CaCl₂. It might be thought that variation in the osmotic pressure might influence these results. But as shown in Fig. 4, the linear relationship between Na and K, in spite of a great variation of osmotic pressure, makes this view rather improbable. The direct proof is hardly possible because, as already shown, non-electrolytes such as dextrose are not indifferent to the muscle but reduce its susceptibility to KCl.

Towards the upper limit determined by the NaCl concentration the antagonisms followed the quantitative rules described above. Much greater concentration of CaCl₂ than that required by our equations had almost no influence. From Table VIII it is apparent that in 0.056 M NaCl the maximal tolerated concentration of KCl increased from 71 M × 10⁻⁴ to 74 M × 10⁻⁴ if greater amounts of CaCl₂ than calculated were used. For the other concentration of NaCl the limiting concentration of KCl was identical no matter whether CaCl₂ was added in calculated amounts or in larger ones. Only in 0.098 M NaCl the limiting KCl concentration was sometimes increased from 108 M × 10⁻⁴ to 113 M × 10⁻⁴ if greater amounts of CaCl₂ were added.

TABLE VIII

Determination of the Maximal Concentration of KCl which can be Balanced by CaCl₂

	If added in calculated amounts			If added in far greater amounts			
M NaCl 0.056	M × 10 ⁻⁴ KCl	71	74	74	74	80	
	M × 10 ⁻⁴ CaCl ₂	69	75	79	162	180	
	Irritability	+	*	-	+	-	
M NaCl 0.071	M × 10 ⁻⁴ KCl	91	97		97	102	113
	M × 10 ⁻⁴ CaCl ₂	83	93		162	162	202
	Irritability	+	-		-	-	-
M NaCl 0.084	M × 10 ⁻⁴ KCl	102	108		108		
	M × 10 ⁻⁴ CaCl ₂	83	95		126		
	Irritability	+	-		-		
M NaCl 0.098	M × 10 ⁻⁴ KCl	108	113		113		
	M × 10 ⁻⁴ CaCl ₂	73	83		122		
	Irritability	+	-		+ or -		

* + = preservation of irritability above the 20 cm. threshold.

- = loss of irritability below the 20 cm. threshold.

V. *The Antagonism Na → Ca*

In order to analyse completely the relationship between Na, K, and Ca, experiments were carried out to decide whether still other

antagonisms exist than those described above. It was also found that CaCl_2 can reduce the irritability of the sartorius muscle if added in a sufficient amount. But, of course, the quantitative difference between the K and the Ca effect is very marked because one needs far larger amounts of CaCl_2 than of KCl in order to reduce the irritability of the muscle. On the other hand, the effective concentration of CaCl_2 depends upon the NaCl concentration in both cases and increases with increasing NaCl concentration (Table IX). While in a 0.042 M NaCl solution only $81 \text{ M} \times 10^{-4} \text{ CaCl}_2$ is required in order to reduce the irritability of the muscle below the 20 cm. threshold, three times as much is necessary in order to exert the same effect in a 0.056 M NaCl solution. The corresponding concentration in a 0.07 M NaCl is far greater, but the latter was not determined because of the interference with an increase of the osmotic pressure.

TABLE IX

The Na → Ca Antagonism. The effect of different CaCl_2 concentrations on irritability.

	$\text{M} \times 10^{-4} \text{ CaCl}_2$				
0.042 M NaCl $5.7 \text{ M} \times 10^{-4} \text{ KCl}$ Irritability	8.1 +	40.5 +	60.75 +	81 —	101.25 —
0.056 M NaCl $5.7 \text{ M} \times 10^{-4} \text{ KCl}$ Irritability	162.0 +	182.25 +	202.5 +	222.75 +	243.0 —
0.071 M NaCl $5.7 \text{ M} \times 10^{-4} \text{ KCl}$ Irritability	299.7 +				

The experiments prove that Na is an antagonist of K and Ca because the effective concentrations of K and Ca increase with increasing concentrations of Na. Experiments of Part II showed that Ca is the antagonist of K, since the effective concentration of K varied in the same direction with varying Ca concentration. It still remains a question whether K also is the antagonist of Ca. If this be the case, it was to be expected that the addition of K could reduce the harmful effect of CaCl_2 or that it would increase the effective concentration of the latter. Neither was the case.

SUMMARY

The irritability of the sartorius muscle of *Rana esculenta* has been studied in different mixtures of NaCl, KCl, and CaCl_2 . The method

permits the detection of very small differences in the KCl effect. Usually the minimal effective KCl and CaCl₂ concentration is determined which leads to a rapid loss in irritability. The dependence of the KCl effect upon the NaCl and the CaCl₂ concentration is represented by a straight line. Concerning the antagonism between Na and K the result is that the ratio Na : K is constant. That does not hold for the antagonism between K and Ca although this also corresponds to a straight line. But in the latter case the relation is represented by the equation $y = ax + b$ and b has a definite value, while in the first case $b = 0$ and therefore $a = y/x$. The quantitative results show that within certain limits 0.014 M NaCl has the same antagonistic value against KCl as 0.00081 M CaCl₂. Therefore, it is possible to predict the behavior of the muscle in any other solution if the antagonism has been investigated for one single mixture of these three salts. The results agree rather exactly with this assumption. There is, however, a very characteristic limit of the validity of these equations. It is found that the NaCl concentration determines the highest KCl concentration, the effect of which can still be offset by the addition of CaCl₂. It is higher the greater the NaCl concentration. Therefore the antagonistic action of Na against K is not only quantitatively but also qualitatively different from the antagonistic effect of Ca against K.

An antagonism between Na and Ca was also found. The complete analysis leads to the qualitative formula: $\begin{matrix} \text{Na} \rightarrow \text{K} \\ \searrow \text{Ca} \nearrow \end{matrix}$ in which the arrow indicates the cation which is able to balance the toxic effect of another cation.

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